

Research Paper



Sensitivity of klebsiella pneumoniae bacteria isolated from the urine of patients with urinary tract infections in kirkuk city/iraq

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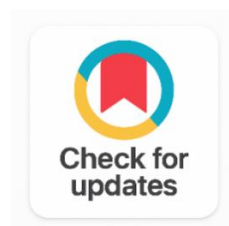
Klebsiella Pneumonia

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ABSTRACT

This study was conducted to investigate the presence of Klebsiella pneumoniae bacteria causing urinary tract infections among patients suffering from urinary infections, to isolate and test their antibiotic sensitivity in order to identify the effective antimicrobial agent against them. A total of 136 urine samples were collected from individuals with urinary tract infections, aged 2 to 75 years, during the period from August 6, 2023, to November 16, 2023. The samples were cultured on MacConkey agar and blood agar. A positive growth was exhibited in 65 samples, accounting for 47%, while 71 samples did not show any bacterial growth, making up 52%. Out of these, 17 isolates were identified as Klebsiella, with a positivity rate of 26%, diagnosed through microscopic, cultivation, and biochemical tests. The study results indicated that Klebsiella pneumoniae was one of the most common pathogenic causes after E.coli bacteria. The Incidence of Klebsiella was higher in females than in males, with most infections occurring in married women more than in single women. Klebsiella bacteria showed a high resistance of 100% against Gentamycin, Rifampicin, Tetracycline, Ampicillin, and Cefoxitin, while the resistance rates varied for Imipenem, Tobramycin, Trimethoprim, with 76%, 52%, and 88% respectively. The sensitivity rates ranged for Chloramphenicol, Ofloxacin, Amikacin, and Azithromycin with 41%, 35%, 11%, and 17.

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1. INTRODUCTION

Urinary tract infection (UTI) is one of the most common infections after respiratory tract infections, considered as a community-acquired infections, and ranks first in hospital-acquired infections. A urinary tract infections can affect any part of the urinary system, including the kidneys, ureters, bladder, and urethra. Most infections affect the lower part of the urinary tract, i.e., the bladder and the urethra. Women are more susceptible to urinary tract infections compared to men. *K. pneumoniae* bacteria are the main cause of urinary tract infection and are characterized as non-motile, lactose-fermenting, and Gram-negative bacteria.

They possess high resistance to many antibiotics such as penicillins, cephalosporins, carbapenems, and aminoglycosides, which leads to a decreased effectiveness of drugs in treating their infections. Resistance may occur due to increased efflux of antibiotics out of the cell by efflux pumps, inactivation of the drug by destruction of the β -lactam ring in β -lactam antibiotics, or by altering the binding to the target site of the antibiotic. Many *K. pneumoniae* strains form biofilms around the bacterium, which further increases their resistance to antibiotics. *K. pneumoniae* bacteria primarily resist antibiotics through five main mechanisms: inactivation of antibiotics by enzymes such as β -lactamase, alteration of the antibiotic target, loss of porin and mutations, extrusion of antibiotics out of the cell by efflux pumps, and formation of biofilms.

Due to the increasing rate of antibiotic resistance, experts from the World Health Organization anticipate that by 2050, the incidences of disease caused by infectious diseases will be similar to those in the "pre-antibiotic era" [1]. Up to 60% of women will experience symptoms of a urinary tract infection at least once during their lifetime. About 10% of women in the United States experience one or more symptomatic urinary tract infections each year. Sexually active young women aged 18-24 years have the highest rates of urinary tract infection. About 25% of these women experience spontaneous resolution of symptoms, and a similar number develop the infection. The prevalence of urinary tract infections in men is much less than in women and primarily occurs in men with structural abnormalities in the urinary tract and in older men [2].

1.1 Pathophysiology

Lower urinary tract infections, also known as cystitis, are more prevalent in women than in men, primarily due to anatomical differences, including a shorter urethral length and the moist periurethral environment in women. Urinary tract infections typically begin with the contamination of the periurethral area by urinary pathogens present in the intestines, followed by colonization of the urinary tract, and finally, the migration of flagella and pili of the pathogen to the bladder or kidney. The adhesion of bacteria to the urinary epithelium is fundamental in causing urinary tract infections.

Infection occurs when the virulence mechanisms of bacteria overcome the effective defense mechanisms of the host. Upper urinary tract infections, also known as pyelonephritis, develop when urinary pathogens ascend to the kidneys via the ureters. Infection can occur when bacteria attach to a urinary catheter, kidney stone, or bladder stone, or when they are trapped in the urinary tract due to physical obstruction. In severe cases of pyelonephritis, the affected kidney may become swollen and abscesses may form on the surface [3].

1.2 Causative Factors of Urinary Tract Infections

In non-pregnant adult women with normal urinary tracts, the bacterial environment rarely develops into symptomatic cystitis or pyelonephritis. Fundamental causative factors for urinary tract infections include diabetes mellitus, intrarenal obstruction associated with kidney stones, nephropathy resulting from uric acid, polycystic kidney disease, nephropathy due to hypokalemia or analgesic abuse, and renal lesions resulting from sickle cell disease. Patients with structural abnormalities are significantly prone to urinary tract infections due to obstruction of urinary flow. Urinary stasis increases susceptibility to infection, and the urinary tract is usually colonized by bacteria, sexual intercourse can propel bacteria into the female bladder [4].

1.3 Treatment of Urinary Tract Infections

The appropriate choice of treatment and treatment duration primarily depends on the patient's medical history, age, chronicity of the illness, as well as the patient's resistance to certain antibiotics. Sensitivity testing can be conducted to assist the physician in prescribing the treatment. When choosing an antibiotic for therapy, it is important to consider the drug's side effects on the patient, such as vomiting and nausea, and the appropriate dosage to eliminate the infection. *K. Pneumoniae* bacteria demonstrate resistance to many commonly used antibiotics, including beta-lactam antibiotics, aminoglycosides, and macrolides.

Resistance occurs due to overuse and indiscriminate use of medicines, and frequent switching between drugs. For example, prescribing antibiotics in viral infections without any sign or evidence of bacterial infections, without taking a sample for culture and sensitivity testing, giving broad-spectrum antibiotics, or without consulting a doctor [5].

2. RELATED WORK

The results of [6] on the antibiotic sensitivity testing for *K. pneumoniae* bacteria against ten types of antibiotics including Ofloxacin, Tetracyclin, Imipenem, Oxacillin, Tobtomyacin, Azithromycin, Amikacin, Cefotaxime, Nitrofurantoin, and Ceftazidime showed that the isolates had 100% resistance to the Tetracyclin antibiotic and multiple resistances to the rest of the antibiotics. Whereas the results of [7] was on eight types of antibiotics tested against *K. pneumoniae* bacteria showed resistance rates to Gentamycin at 93% and to Ampicillin at 83%.

The results of the study [8] on ten commonly used antibiotics against *K. pneumoniae* isolated from urinary tract infections in children in the city of Kirkuk showed high resistance to beta-lactam antibiotics (penicillins) while exhibiting high sensitivity towards Quinolones. These findings are consistent with the results of our current study.

3. METHODOLOGY

3.1 Sample Collection

A total of 136 urine samples were collected from the period of 06 /August /2023 to 16 /November /2023 from inpatients and outpatients at Azadi Teaching Hospital, Daquq General Hospital, and some private laboratories in the city of Kirkuk and the district of Daquq. The ages of the individuals ranged from 2 to 75 years, who presented with symptoms of urinary tract infections. Mid-stream urine samples were then collected in sterile, tightly sealed glass bottles, and information for each patient was recorded on a dedicated questionnaire form.

3.2 Urine Culture

A drop from each urine sample was taken using a loop and inoculated onto blood agar and MacConkey agar media, then incubated at 37°C for 24 hours to obtain pure colonies.

3.3 Isolation and Identification of *K. Pneumoniae* Based on Cultivation Characteristics

After the colonies grown on MacConkey and blood agar media were observed, they were preliminarily diagnosed based on colony size, color, shape, and edges.

3.4 Microscopic Examination of Isolates

A smear was made from an individual bacterial colony from each culture using a sterile loop, placed on a clean glass slide, then heat-fixed and stained with Gram stain. Once the slide dried, it was examined microscopically under an oil-immersion lens to observe the Gram characteristic, as well as the shape, size, and arrangement of the bacterial cells.

3.5 Biochemical Tests

The bacterial isolates under study were identified by performing several biochemical tests, which are: Oxidase, catalase, indole, methyl red, Voges-Proskauer, citrate utilization, Coagulase, mannitol fermentation, and motility.

3.6 Antibiotic Susceptibility

An antibiotic sensitivity test for the bacterium *K. pneumoniae* was conducted using the disk diffusion method on Muller-Hinton agar according to the [9] method. The results were read by observing the inhibition zones around the antibiotic disks. Table 1 shows the antibiotics used in the study along with their inhibition diameters.

Table 1. Shows the Antibiotics Used in this Study

No.	Antibiotics	Antibiotics Symbols	Concentration / μg	(mm)Diameter of Inhibition Zones		
				R	I	S
1	Tetracycline	TE	10 μg	≤ 11	12-14	≥ 15
2	Ampicillin	AMP	10 μg	≤ 13	14-16	≥ 17
3	Cefoxitin	FOX	30 μg	≤ 14	17-15	≥ 18
4	Imipenem	IMP	10 μg	≤ 19	20-22	≥ 23
5	Amikacin	AK	10 μg	≤ 12	19-17	≥ 20
6	Rifampicin	RA	5 μg	≤ 16	17-19	≥ 20
7	Azithromycin	AZM	15 μg	≤ 12	-	≥ 13
8	Chloramphenicol	C	30 μg	≤ 12	17-13	≥ 18
9	Trimethoprim	TMP	10 μg	≤ 10	11-15	≥ 16
10	Gentamicin	CN	10 μg	≤ 14	15-17	≥ 17
11	Ofloxacin	OF	5 μg	≤ 12	13-15	≥ 16
12	Tobromycin	TOB	10 μg	≤ 12	13-16	≥ 17

4. RESULTS AND DISCUSSION

4.1 Isolation of *K. Pneumoniae*

The current study included 136 urine samples, out of which 60 samples showed bacterial growth. The number of isolates belonging to *K. pneumoniae* was 17, constituting 28%. Meanwhile, 71 samples did not show any bacterial growth.

4.2 Cultivation Characteristics

The results of the cultivation diagnosis revealed mucoid, shiny pink colonies on MacConkey Agar that ferment lactose, while the colonies on blood agar were transparent and glossy, with no hemolysis [10].

4.3 Microscopic Characteristics

The results of the microscopic examination showed that 17 isolates were rod-shaped and encapsulated, appearing singly, in pairs, and in short chains. To differentiate them from other species, biochemical tests were relied upon.

4.4 Biochemical Tests

Biochemical tests were conducted on isolates belonging to the genus *Klebsiella*, identifying them as *pneumoniae* based on its morphological, microscopic features, and biochemical tests listed in Table 2. The IMVIC test was also performed to confirm the diagnosis of the isolates. The results were negative for the indole and methyl red tests and positive for the Voges-Proskauer and citrate utilization tests [11].

Table 2. Biochemical Tests

Biochemical Tests	K.Pneumoniae
Gram Stain	-
Catalase	+
Indole	-
Methyl red	-
Voges -Proskauer	+
Citrate utilization	+
Oxidase	-
Motility	-

4.5 Antibiotic Susceptibility Test

Table 3 shows the percentage of sensitivity and resistance of 17 isolates of Gram-negative *Klebsiella pneumoniae*, which were isolated from patients with urinary tract infections, to 12 antibiotics according to the Kirby-Bauer method. Sensitivity and resistance were determined based on the inhibition zone of antibiotic disks used in the study and compared with the standards in [12]. All isolates showed 100% resistance to Gentamycin, which is similar to the study by [7] with a resistance rate of 95%, close to the findings by [13] at 83%, and in line with the results by [14]. The resistance is attributed to the absence of receptor sites on the ribosome or enzymatic degradation of the antibiotic controlled by plasmids or the loss of efficient transport into the bacteria, which is chromosomally controlled [15]. The Isolates also demonstrated a high resistance rate of 100% to Ampicillin, which is close to the results by [7] at 96%, consistent with [16] at 100%, and does not align with the findings by [17], at 75%. This is due to the degradation of the antibiotic by the beta-lactamase enzyme produced by the bacteria [15].

Resistance to Tobramycin was variable at 52%, and there was a 100% resistance rate to Tetracycline, which corresponds with the studies by [8], [18], [16]. Resistance to aminoglycosides is due to chromosomal mutations, such as in the gene encoding the target protein in the 30S ribosomal subunit [15]. Amikacin showed a resistance rate of 35%, which is close to the findings by [16] but does not match the results by [6], [18]. The sensitivity of the antibiotic is due to its success in reaching the target by inhibiting protein synthesis via inhibition of the 30S subunit [15]. The resistance rate for Imipenem was 88%, which does not match the results by [6], [17], but is close to [13].

The resistance arises from the destruction of the antibiotic by beta-lactamase enzymes [19]. For the antibiotic Cefoxitin, the resistance rate was 100%, aligning with the finding by [20], but not with the findings by [17]. It also approximates the results by [13], and resistance is due to the production of cephalosporinase enzyme [21]. The isolates showed a resistance to Trimethoprim at 76%, which matches the study by [20] and is close to the results by [8], [22]. The resistance arises from the absence of ribosomal receptor sites or enzymatic degradation of the treatment controlled by plasmids [23]. As for Chloramphenicol, the resistance rate was 17%, which does not align with the study by [20] but is close to the findings by [22], [13]. Chloramphenicol inhibits protein synthesis by preventing the bonding of amino acids to the peptide chain on the 50S subunit by interfering with the action of peptidyl transferase [15]. Azithromycin showed a resistance rate of 41%, aligning with the results by [7], but not with those by [13], [16]. This medium level of resistance allows for its use in treatment and is due to the antibiotic inhibiting protein synthesis by irreversibly binding to the 50S subunit [15].

Regarding the antibiotic Rifampicin, all isolates were resistant to it at a rate of 100%. This finding is similar to the research by [24] and is consistent with [18], [22]. The resistance to this antibiotic is due to genetic mutations in the Catalase peroxidase gene. Meanwhile, the isolates showed a low resistance to Ofloxacin at a rate of 11%, which is close to the study by [20], and is 100% consistent with the findings by [13], [22]. Since this represents a high sensitivity rate, it can be considered an appropriate antibiotic for the treatment of urinary tract infections. This antibiotic works by inhibiting DNA synthesis through the inhibition of the DNA gyrase.

Table 3. The Percentage of Sensitivity and Resistance of Klebsiella Pneumonia

No	Antibiotics	Resistant Isolates		Sensitive Isolates	
		Number	Percentage%	Number	Percentage %
1	Imipenim	15	88%	2	11%
2	Ofloxacin	2	11%	15	88%
3	Gentamcin	17	100%	0	0%
4	Amikacin	6	35%	11	64%
5	Azethromycin	7	41%	10	58%
6	Ampicilin	17	100%	0	0%
7	Cefoxitin	17	100%	0	0%
8	Tobromycin	9	52%	8	47%
9	Chloramphenicol	3	17%	14	82%
10	Rifampicin	17	100%	0	0%
11	Trimethoprim	13	76%	4	23%
12	Tetracyclin	17	100%	0	0%

5. CONCLUSION

The incidence rate is higher in females than in males, and in married women more than in single ones. The bacterial isolates varied in their sensitivity and resistance to antibiotics, reflecting a significant diversity in the characteristic of resistance to the antibiotics used and their possession of different resistance mechanisms.

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Author Contributions Statement

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Shukran Abdul Hussein Mohammed	✓	✓	✓	✓		✓		✓	✓	✓	✓			✓
Najdat Bahjat Mahdi	✓		✓		✓		✓		✓		✓		✓	
Hussain Salih Akbar		✓		✓		✓		✓		✓		✓		✓

C : Conceptualization

M : Methodology

So : Software

Va : Validation

Fo : Formal Analysis

I : Investigation

R : Resources

D : Data Curation

O : Writing - Original Draft

E : Writing - Review & Editing

Vi : Visualization

Su : Supervision

P : Project Administration

Fu : Funding Acquisition

Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Informed Consent

All participants were informed about the purpose of the study, and their voluntary consent was obtained prior to data collection.

Ethical Approval

The study was conducted in compliance with the ethical principles outlined in the Declaration of Helsinki and approved by the relevant institutional authorities.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.



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